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Predictions in the Face of Clinical Reality: *HistoCheck* versus High-Risk HLA Allele Mismatch Combinations Responsible for Severe Acute Graft-versus-Host Disease

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HLA polymorphism remains a major hurdle for hematopoietic stem cell transplantation (HSCT). In 2004, Elsner et al. proposed the *HistoCheck* Web-based tool to estimate the allogeneic potential between HLA-mismatched stem cell donor/recipient pairs expressed as a sequence similarity matching (SSM). SSM is based on the structure of HLA molecules and the functional similarity of amino acids. According to this algorithm, a high SSM score represents high dissimilarity between MHC molecules, resulting in a potentially more

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deleterious impact on stem cell transplant outcomes. We investigated the potential of SSM to predict high-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease (aGVHD grades III and IV) published by Kawase et al., by comparing SSM in low- and high-risk combinations. SSM was calculated for allele mismatch combinations using the *HistoCheck* tool available on the Web (www.histocheck.org). We compared ranges and means of SSM among high-risk (15 combinations observed in 722 donor/recipient pairs) versus low-risk allele combinations (94 combinations in 3490 pairs). Simulation scenarios were created where the recipient's HLA allele was involved in multiple allele mismatch combinations with at least 1 high-risk and 1 low-risk mismatch combination. SSM values were then compared. The mean SSM for high- versus low-risk combinations were 2.39 and 2.90 at A, 1.06 and 2.53 at B, 16.60 and 14.99 at C, 4.02 and 3.81 at DRB1, and 7.47 and 6.94 at DPB1 loci, respectively. In simulation scenarios, no predictable SSM association with high- or low-risk combinations could be distinguished. No DQB1 combinations met the statistical criteria for our study. In conclusion, our analysis demonstrates that mean SSM scores were not significantly different, and SSM distributions were overlapping among high- and low-risk allele combinations within loci HLA-A, B, C, DRB1, and DPB1. This analysis does not support selecting donors for HSCT recipients based on low *HistoCheck* SSM scores.

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KEY WORDS: HLA, Mismatched, Unrelated donor, HSCT, *HistoCheck*

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapy for many hematologic and nonhematologic disorders. The steady expansion of unrelated stem cell donor registries has facilitated finding a matched donor for many transplant candidates, particularly those with common human leukocyte antigens (HLA) alleles and haplotypes. However, the extensive polymorphism of HLA and the remarkable disparity in the distribution of alleles and haplotypes among individuals of different ethnic and racial backgrounds remain a major hurdle for access of many patients to HSCT. A number of studies have shown that donor/recipient matching for alleles at HLA-A, -B, -C, -DRB1, and -DQB1 loci lowers the risk of clinically severe acute graft-versus-host disease (aGVHD) [1-3]. Recently, HLA-DPB1 allele mismatches were also significantly associated with an increased incidence of GVHD [4-6]. When only HLA mismatched donors are available for a given patient, the challenge becomes determining which mismatch has a less deleterious impact on clinical outcomes. Bray and colleagues [7], in a comprehensive commentary, described the National Marrow Donor Program (NMDP) guidelines for unrelated HSC donor selection including the impact of mismatches at different loci on HSCT clinical outcomes. In 2004, Elsner and colleagues [8] proposed the *HistoCheck* Web-based tool to estimate the allogenicity of mismatches with a sequence similarity matching (SSM) concept. In this concept, an SSM score (ie, allogenicity index) is generated by rating the amino acid (AA) differences between HLA allelic products based on the position within the HLA molecule and the functional similarity of AA within proteins [9]. A high SSM score (also referred to as Dissimilarity Score [DSS]) represents high dissimilarity between HLA alleles resulting in a potentially greater deleterious impact on clinical outcomes. However, this algorithm has been challenged by 2 single-center analyses that could not associate higher SSM scores with aGVHD (in 26 patients) or in vitro T cell reactivity (in 74 patients) [10,11]. In the present study, we investigated the potential of SSM scores to predict high-risk HLA allele mismatch combinations responsible for severe aGVHD (grades III and IV) observed in a large cohort (5120 consecutive patients) of HSCT donor/recipient pairs. These allele combinations were observed in HSC transplants facilitated by the Japan Marrow Donor Program (JMDP) and published by Kawase et al. [12]. This investigation was conducted by comparing SSM scores in high-risk and low-risk allele combinations at HLA-A, -B, -C, -DRB1, and -DPB1 loci. No high-risk allele combinations at DQB1 locus met the predetermined level of statistical significance ($P < .005$) and thus SSM predictions were not evaluated in this study.

METHODS

Identification of High- and Low-Risk HLA Allele Mismatch Combinations

Significant high-risk HLA allele mismatch combinations were identified by retrospective analysis of 5210 consecutive registered patients who underwent transplantation through the JMDP. Patient characteristics, HLA matching and typing methods, and transplant procedures are described elsewhere [12]. Briefly, 15 mismatch allele combinations were identified as high-risk allele mismatch combinations (4 at HLA-A, 1 at HLA-B, 6 at HLA-C, 1 at HLA-DRB1, and 2 at HLA-DPB1 loci). Only 1-allele mismatched pairs in the same HLA locus were considered, and

adjusted by HLA locus matching in the other loci. These mismatch combinations were found to be associated with hish risk of severe aGVHD in a multivariable Cox regression model constructed with mismatch combinations and potential confounders. Confounders considered were sex, patient age, donor age, type of disease, risk of leukemia relapse, GVHD prophylaxis, and preconditioning. Each HLA mismatch combination was evaluated for each locus separately, for example, in the A*02:06-A*02:01 allele mismatch combination, the donor has HLA-A*02:06, recipient has HLA-A*02:01, and the other HLA-A allele of each donor and recipient was identical. This mismatch was compared with the HLA-A allele match. An allele mismatch combination was designated as a significant high-risk combination for severe aGVHD based on *P* values for hazard ratios (HR) for developing severe aGVHD of <.005. For example, the above mismatch combination was observed in 131 donor/recipient pairs and was associated with increased hazard of severe aGVHD (HR: 1.78, 95% confidence interval [CI]: 1.32-2.41, $P < .001$). Therefore, this was considered a high-risk mismatch allele combination. On the other hand, allele mismatch combinations with a 95% CI of the HR including 1.00 were considered low-risk combinations. For example, the combination A*24:02-A*24:20 was observed in 60 donor/recipient pairs and was not associated with increased risk of severe aGVHD (HR: 0.64, 95% CI: 0.32-1.30, $P = .225$). In addition, high-risk allele combinations observed in the context of 2 loci-linked mismatches such as (DRB1*14:03-DQB1*03:01) – (DRB1*14:01-DQB1*05:02) were excluded from this analysis because there are no explicit provisions regarding the utility of the *HistoCheck* algorithm in this setting.

SSM Score Calculation and Comparisons

SSM scores were calculated using the *HistoCheck* tool available online at http://www.histocheck.org/ according to the instructions posted on that Website. The averages and the distribution of SSM values were compared among high- and low-risk allele mismatch combinations in the same locus.

Simulation Scenarios

Hypothetical scenarios were created where the recipient's HLA allele was involved in multiple allele mismatch combinations with at least 1 high-risk mismatch and 1 low-risk mismatch. These scenarios were created to simulate clinical scenarios where multiple mismatched donors are considered for a given recipient in the absence of a matched donor. In these instances, SSM scores were compared among donors from both types of mismatch combinations. In addition, to investigate the impact of the direction of the allele mismatch, we assessed whether any of the identified 15 high-risk mismatch allele combinations associated with severe aGVHD were also a high-risk combination when considering the reverse direction of the mismatch between donor and recipient. For example, in the high-risk mismatch combination HLA-A*26:01/26:02, the recipient had the HLA-A*26:02 allele and the donor had the HLA-A*26:01. In this instance, we reviewed the list of the high- and low-risk mismatch allele combinations from the Kawase study to assess the risk associated with the reverse combination (ie, recipient is HLA-A*26:01 and donor is HLA-A*26:02). The same assessment was performed for all identified high-risk combinations.

Statistical Analysis

For the purpose of this analysis, low-risk mismatch allele combinations were used as a control group for SSM comparisons. SSM means and distributions were compared among aggregates of low-risk mismatch allele combinations and aggregates of high-risk combinations as well as individual high-risk combinations.

RESULTS

High- versus Low-Risk Allele Mismatch Combinations

In the HLA-A locus, the 4 high-risk allele mismatch combinations (observed 214 HSCT donor-recipient pairs) had a mean SSM of 2.39 (range: 1.04-4.30) compared to a mean SSM of 2.90 (range: 1.04-5.66) in 11 low-risk combinations (observed in 389 pairs). Individual high-risk combinations had the following SSM values: 1.04 (A*02:06-02:01), 2.87 (A*02:06-02:07), 1.36 (A*26:02-26:01), and 4.3 (A*26:03-26:01).

The 1 high-risk combination (B*15:01-15:07, observed in 19 pairs) at HLA-B locus had an SSM value of 1.06. The mean SSM for 4 B low-risk combinations was 2.53 (range: 1.74-2.81). In the HLA-C locus, the mean SSM for the 7 high-risk combinations (observed in 316 pairs) was 16.60 (range: 12.36-23.86) compared to a mean SSM of 14.99 (range 1.52-23.86) in 18 low-risk combinations (observed in 578 pairs). The 1 high-risk combination (DRB1*04:05-04:03, observed in 53 pairs) at HLA-DRB1 locus had an SSM value of 4.02. The mean SSM for 4 DRB1 low-risk combinations was 3.81 (range: 1.30-10.41). Finally, at the HLA-DPB1 locus, the mean SSM for the 2 high-risk combinations (observed in 120 pairs) was 7.47 (range: 6.98-7.95) compared to a mean SSM of 6.94 (range 1.21-12.87) in 36 low-risk combinations (observed in 1594 pairs).

A graphical representation of overall comparisons of SSM means and distributions among aggregates of low-risk mismatch allele combinations and aggregates of high-risk combinations as well as individual high-risk combinations across all loci is depicted in Figure 1. Without exception, in all these loci, the SSM

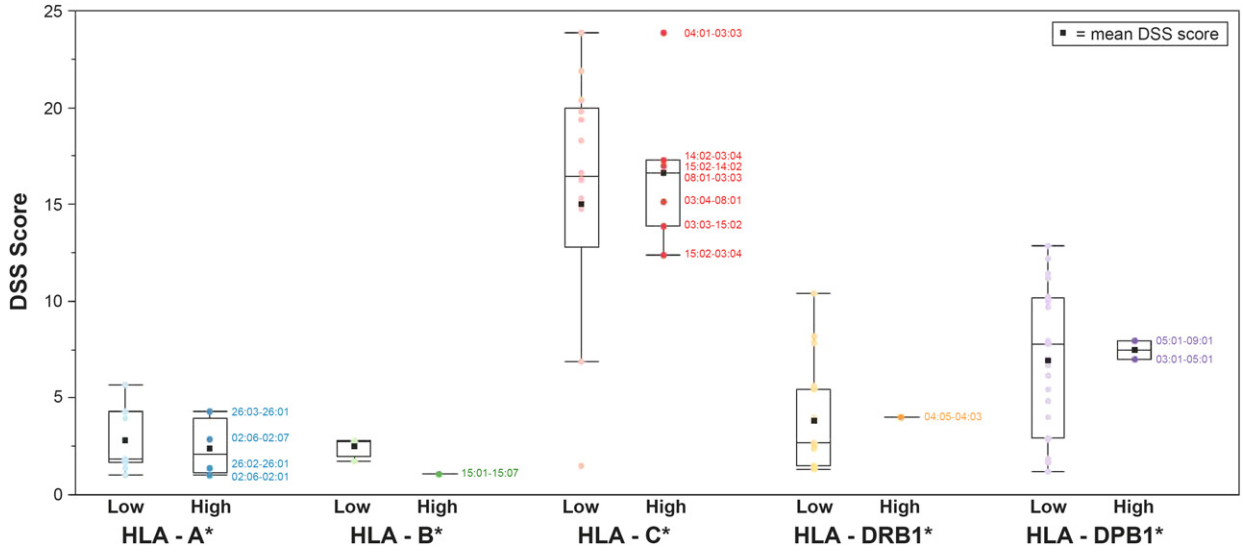

Figure 1. Comparison of SSM in high- versus low-risk mismatch allele combinations. SSM means are not significantly different, and SSM distributions are overlapping among high- and low-risk allele combinations within loci HLA-A, -B, -C, -DRB1, and -DPB1.

score for each high-risk combination fell within or below the range for the low-risk combinations. Two of 15 high-risk combinations had SSM scores that were lower than or equal to the lowest SSM score in any of the low-risk combinations in the corresponding locus. A detailed list of individual low-risk combinations is presented in the original publication by Kawase et al. [12].

Simulation Scenarios

To simulate clinical scenarios where multiple mismatched donors are considered for a given recipient, SSM values for high-risk and low-risk combinations including the same recipient allele were compared. The recipient allele in 13 of the 15 identified high-risk mismatch allele combinations (Loci HLA*A, C*, DRB1*, and DPB1*) was included in 1 or more low-risk allele mismatch combinations (Table 1). In 1 scenario, a recipient with an A*02:07 allele had 2 potential mismatched donors at this allele for either A*02:06 (high-risk mismatch allele combination) or A*02:01 (low-risk mismatch allele combination). SSM values for the high-risk and the low-risk combinations were 2.90 and 1.83, respectively, as one might expect. However, in another scenario, a recipient with an A*02:01 allele had 2 potential mismatched donors at this allele for either A*02:06 (high-risk mismatch allele combination) or A*02:07 (low-risk mismatch allele combination), the opposite was observed. SSM values for the high- and the low-risk combinations were 1.04 and 1.83, respectively. All simulation scenarios are summarized in Table 1.

Overall, no predictable SSM pattern of association with high- or low-risk mismatch allele combinations could be distinguished. In all but 2 instances (at HLA-A* and -DRB1* loci), at least 1 low-risk combination scored a higher SSM value than the high-risk mismatch combination including the same recipient allele.

Direction of the Allele Mismatch Combinations

None of the 15 high-risk combinations were high risk in both directions. In 10 of these combinations, when the direction of the allele mismatch was reversed between donor and recipient, there was no longer an association with severe aGVHD (Table 2). An example of such a unidirectional risk is the combination where the recipient has A*02:01 and the donor has A*02:06 (high risk) versus the recipient having A*02:06 and the donor having A*02:01(low risk). Both of these combinations have an SSM value of 1.04 according to *HistoCheck*.

DISCUSSION

Optimal effectiveness and safety of HSCT requires high degree of HLA allele matching between donors and recipients [7,13]. However, many patients who need HSCT do not have an HLA-matched donor, which lowers the probability of cure. Undoubtedly, a predictive algorithm for definition of "low-risk" HLA mismatches has the potential of broadening the use of mismatched donors and increasing the availability of unrelated donors. Several approaches have been proposed to identify such low-risk mismatches, with the simplest being a comparison between alleles based on the number of amino acid mismatches. However, prior reports have not found any evidence for selecting an allele with a lower number of AA substitutions of its allelic product [14,15]. Although hypothetically appealing, the notion that the lower the number of AA substitutions the more low-risk the mismatch is

Table 1. Simulation Scenarios for Comparison between SSM Scores among Multiple High- versus Low-Risk Mismatched Donors Potentially Available for a Patient with a Given HLA Allele

HLA Locus	MM Combination (Donor-Patient)	N	HR (95% CI)	*P*	Risk Group	SSM
A	02:06-02:01	131	1.78 (1.32-2.41)	<.001	High	1.04
	02:07-02:01	20	1.12 (0.42-3.02)	.81	Low	1.83
	02:06-02:07	27	3.45 (2.09-5.70)	<.001	High	2.90*
	02:01-02:07	28	0.83 (0.34-2.03)	.70	Low	1.83
C	04:01-03:03	42	2.81 (1.72-4.60)	.001	High	23.86
	08:01-03:03	80	2.32 (1.58-3.40)	.001	High	16.65
	07:02-03:03	18	2.16 (0.96-4.85)	.06	Low	21.90
	03:04-03:03	62	0.83 (0.41-1.68)	.61	Low	1.52
	03:04-08:01	69	2.34 (1.55-3.52)	.001	High	7.13
	03:03-08:01	76	1.07 (0.63-1.84)	.78	Low	16.65
	14:02-03:04	23	3.66 (2.00-6.68)	.001	High	17.30
	15:02-03:04	27	3.77 (2.20-6.47)	.001	High	12.36
	08:01-03:04	47	1.64 (0.98-2.76)	.06	Low	15.13
	01:02-03:04	12	1.85 (0.59-5.81)	.29	Low	18.30
	07:02-03:04	33	1.22 (0.58-2.59)	.59	Low	20.38
	03:03-03:04	83	1.08 (0.63-1.85)	.76	Low	1.52
	03:03-15:02	25	3.22 (1.75-5.89)	.001	High	13.88
	08:01-15:02	36	1.59 (0.79-3.21)	.19	Low	15.33
DRB1	04:05-04:03	53	2.13 (1.28-3.53)	.003	High	4.02*
	04:10-04:03	17	1.01 (0.32-3.21)	.98	Low	2.50
	04:06-04:03	30	0.99 (0.46-2.10)	.99	Low	1.45
DPB1	05:01-09:01	71	2.03 (1.30-3.16)	.002	High	7.95
	04:02-09:01	17	0.33 (0.04-2.36)	.27	Low	11.19
	02:01-09:01	47	1.37 (0.75-2.51)	.30	Low	9.98
	03:01-09:01	15	0.8 (0.19-3.22)	.75	Low	3.98
	04:01-09:01	11	0.9 (0.22-3.66)	.89	Low	12.87
	03:01-05:01	49	2.41 (1.49-3.89)	<.001	High	6.98
	06:01-05:01	13	2.5 (0.92-6.77)	.07	Low	7.95
	04:02-05:01	79	1.47 (0.90-2.40)	.12	Low	6.70
	02:02-05:01	41	0.43 (0.13-1.35)	.15	Low	6.18
	09:01-05:01	48	0.71 (0.29-1.73)	.46	Low	7.95
	04:01-05:01	29	0.73 (0.23-2.29)	.59	Low	5.46
	14:01-05:01	26	1.17 (0.48-2.84)	.73	Low	7.77

N indicates number of donor-patient pairs in whom the mismatch allele combination was observed; HR, hazard ratio of developing severe acutt graft-versus-host disease (aGVHD) compared to matched pairs as described in Kawase et al. [12]; SSM, sequence similarity matching; CI, confidence interval.
P values: for the corresponding estimated hazard ratio.
In all but 2 instances (marked with *), at least 1 low-risk combination scored a higher SSM value than the high-risk mismatch combination including the same recipient allele.
*The only 2 instances where SSM value was highest for the combination associated severe aGVHD.

challenged by 2 lines of evidence. First, a recent NMDP registry analysis suggested no difference in outcome when comparing antigen mismatch to allele level (high resolution) mismatch within the same antigen group [13]. Second, at least 2 reports have indicated that severe aGVHD can occur in the presence of only 1 AA mismatch between the donor and recipient allelic products [12,16].

In addition, at least 2 "epitope" based approaches have been proposed. One is based on serologically crossreactive groups (CREG) of antigens, and the other is based on comparisons of "functional epitope" structure through molecular viewing of the HLA structure and the determination of antibody-accessible polymorphic AAs (HLAMatchmaker) [17-19]. Neither of these 2 approaches have yielded predictions that were associated with a survival benefit in patients who underwent mismatched hematopoietic cell transplants from unrelated donors in registry analyses [20,21].

In the current analysis, we investigated whether *HistoCheck* SSM scores can predict 15 high-risk HLA allele mismatch combinations responsible for severe aGVHD observed in 5120 consecutive HSCT donor-recipient pairs facilitated through JMDP. It is noteworthy that the association between these 15 allele combinations and high risk for severe aGVHD has not yet been validated in an independent patient population; however, we believe that this association is reasonably robust because of the conservative approach and the rigorous statistical methods pursued in assigning these associations as described elsewhere [12]. SSM score comparisons were performed between high-risk and low-risk combinations at loci HLA-A, -B, -C, -DRB1, and -DPB1. Significant overlap exists between the high- and low-risk mismatches with respect to SSM scores. In all investigated loci, the SSM score for each high-risk combination fell within or below the range for the low-risk combinations, thus demonstrating the unreliability of SSM scores with respect to aGVHD risk. In addition, in 2 high-risk combinations (13%), SSM scores were less than or equal to the lowest SSM score in any of the low-risk combinations in the

Table 2. Mismatch Allele Combinations Where the Risk of aGVHD Depends on the Direction of the Mismatch

HLA Locus	MM Combination (Donor-Patient)	N	HR (95% CI)	*P*	Risk Group	SSM
A*	02:06-02:01	131	1.78 (1.32-2.41)	<.001	High	1.04
	02:01-02:06	138	1.23 (0.87-1.73)	.223	Low	
	02:06-02:07	27	3.45 (2.09-5.70)	<.001	High	2.87
	02:07-02:06	22	0.71 (0.23-2.24)	.571	Low	
	26:02-26:01	21	3.35 (1.89-5.91)	<.001	High	1.36
	26:01-26:02	24	0.64 (0.26-1.58)	.34	Low	
	26:03-26:01	35	2.17 (1.29-3.64)	.003	High	4.30
	26:01-26:03	34	1.37 (0.73-2.57)	.326	Low	
C*	08:01-03:03	80	2.32 (1.58-3.40)	.001	High	16.65
	03:03-08:01	76	1.07 (0.63-1.84)	.782	Low	
	03:04-08:01	69	2.34 (1.55-3.52)	.001	High	15.13
	08:01-03:04	47	1.64 (0.98-2.76)	.057	Low	
	04:01-03:03	42	2.81 (1.72-4.60)	.001	High	23.86
	03:03-04:01	31	1.73 (0.89-3.36)	.103	Low	
DRB1*	04:05-04:03	53	2.13 (1.28-3.53)	.003	High	4.02
	04:03-04:05	54	1.27 (0.74-2.20)	.379	Low	
DPB1*	05:01-09:01	71	2.03 (1.30-3.16)	.002	High	7.95
	09:01-05:01	48	0.71 (0.29-1.73)	.457	Low	
	03:01-05:01	49	2.41 (1.49-3.89)	<.001	High	6.98
	05:01-03:01	83	1.20 (0.75-1.94)	.434	Low	

N indicates number of donor-patient pairs in whom the mismatch allele combination was observed; HR, hazard ratio of developing severe acute graft-versus-host disease (aGVHD) compared to matched pairs as described in Kawase et al. [12]; SSM, sequence similarity matching; CI, confidence interval.
P values: for the corresponding estimated hazard ratio.
None of the 15 high-risk combinations were high risk in both directions. In 10 of these combinations, when the direction of the allele mismatch was reversed between donor and recipient, there was no longer an association with severe aGVHD.
*The only 2 instances where SSM value was highest for the combination associated with severe aGVHD.

corresponding locus. Furthermore, when simulation scenarios were constructed for recipients included in both high- and low-risk combinations, one would expect that high-risk mismatch combinations have higher SSM values compared to low-risk combinations of the same recipient allele. However, only 2 of the 15 high-risk combinations (13%) had higher SSM scores than alternative low-risk combinations potentially available for a patient with alleles included in these combinations. Interestingly, none of the identified 15 mismatch allele combinations were associated with high-risk for severe aGVHD in both directions. Ten of these combinations were no longer associated with severe aGVHD when the direction of the allele mismatch was reversed between the donor and the recipient. However, the SSM score remains unchanged bcause *HistoCheck* does not distinguish the direction of the allele mismatch between donor and recipient.

The lack of association between SSM and clinical data in our study may be attributed at least in part to the limitations acknowledged by the developers of the algorithm. Namely, underestimating the impact of single AA mismatches, not accounting for clinical data suggesting disproportionately larger impact of substitutions at given positions, particularly position 116, and assignment of positions based on crystallographic data of HLA-A2 for class I and of HLA-DR1 for class II alleles [8]. In addition, *HistoCheck* by design does not account for the direction of the allele mismatch, which has shown to be clinically significant [12].

Limitations of our study include relatively few numbers of high-risk allele mismatch combinations, not including mismatch combinations at the HLA-DQB1 locus, the potential for misclassification of some mismatch combinations as low risk because of relatively small number of subjects in subcategories, and inclusion of patients only from 1 ethnic background. Nevertheless, these results and previous reports do not support the utilization of *HistoCheck* predictions in unrelated donor selection [10,11].

In conclusion, safely maximizing access to mismatched HSCT unrelated donors requires a robust understanding of the rules that govern permissible HLA mismatching, and a better understanding of HLA-associated risks of GVHD. The lack of association between the *HistoCheck* predictions and all previously proposed prediction algorithms and the observed clinical outcomes strongly emphasizes the utmost importance of clinical validation of any prediction algorithm prior to its utilization in clinical patient care. Moving forward, it is prudent in evaluating any prediction algorithm to rely primarily on clinical correlations rather than simply putative biological plausibility.

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